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TITLE: Multispecies, Integrative GWAS for Focal Segmental Glomerulosclerosis

PRINCIPAL INVESTIGATOR: Simone Sanna-Cherchi

RECIPIENT: TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF New York
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14. ABSTRACT

Primary idiopathic nephrotic syndrome (NS) caused by focal segmental glomerulosclerosis (FSGS) or minimal change disease (MCD) is a frequent cause of end-stage renal disease (ESRD. We investigated the genetic basis of FSGS and recruited a heterogeneous population of Caucasian descent ascertained for FSGS (88% of the cases) and MCD (12%), for a total of 1,153 cases. A set of independent and meta-analyzed case-control, genome-wide association studies (GWAS) were performed using an additive model with covariate-correction for population stratification in Quality control assessment was carried out according to standard practices. In a Meta-analysis of three, combined Caucasian cohorts (1153 cases, 2393 controls), a significant association was found for the SNP rs28383303 (OR=1.57, 95%CI: 1.29-1.67, P= $1.48x10^{-8}$). The variant was identified in a 50kb haploblock on chromosome 6p21, which contains the gene encoding the HLA complex class II HLA-DQ alpha chain 1 (*HLA-DQA1*). In line with previously reported findings implicating the HLA system in childhood-onset nephrotic syndrome and membranous nephropathy, our results indicate the association of HLA risk alleles with NS in individuals of Caucasian descent. Our findings allude to a role for HLA in modulating adaptive immunity and suggest a basis for understanding the complex genetic mechanisms of FSGS.

15. SUBJECT TERMS FSGS,
MCD, GWAS, CNV

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

ing.

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific aim 1: A Genome-wide association study for common single nucleotide polymorphisms and rare copy number variations in 7,559 FSGS and over 50,000 controls.

1a. Genotyping of 7,559 FSGS patients with the Illumina Global MEGA Power Chip.

Sample Collection, preparation and genotyping is ongoing and anticipated to be completed by December 2017

1b. CNV burden analysis and annotation of deleterious structural variants

In progress

1c. Joint-cohorts genome-wide association study

Preliminary analysis completed by July 2017. A comprehensive GWAS is to be completed by March 2018.

Specific aim 2: A GWAS for FSGS in a mouse leveraging the power of the newly developed DO strains.

See progress report from Dr Gharavi

Specific aim 3. Cross annotation between human and mouse GWAS and identification of downstream dysregulated pathways and networks.

This aim will be developed mainly at the end of year 2 and during year 3 of fund

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to

Specific aim 1: A Genome-wide association study for common single nucleotide polymorphisms and rare copy number variations in 7,559 FSGS and over 50,000 controls.

1. We are currently in the process of genotyping clinically ascertained nephrotic syndrome (FSGS and MCD) samples to increase the overall GWAS and CNV analysis cohort size according to the project milestones and goals.

2. We conducted an interim analysis on the initial ancestrally and phenotypically heterogeneous cohort of Nephrotic syndrome for a total of 1,957 cases and 57,327 controls (**Table 1**). Using a principal component analysis (PCA), guided by samples from the 1000 genomes project, to genetically infer ancestry (**Figure 1**), three cohorts of Caucasian descent (Western European: 301, Italian: 754, Turkish: 98, Total: 1,153) were selected for subsequent GWA analysis (**Table 2**). An ethnically matched control cohort of 2,392 individuals was also selected. A preliminary GWAS was conducted in this cohort of FSGS (88% of the cases) and MCD (12%), for a total of 1,153 cases.

   A set of independent and meta-analyzed case-control, genome-wide association studies (GWAS) were performed using an additive model with covariate-correction for population stratification in three cohorts of Caucasian descent (Western European: 301, Italian: 754, Turkish: 98), matched genetically with 2,393 controls. Quality control assessment was carried out according to standard practices. In a Meta-analysis of three, combined Caucasian cohorts (1153 cases, 2393 controls), a significant association was found for the SNP rs28383303 (OR=1.57, 95%CI: 1.29-1.67, *P*= 1.48×10^{-8}) (**Table 3** and **Figure 2**). All three cohorts contributed to the signal without evidence for heterogeneity. The variant was identified in a 50kb haploblock on chromosome 6p21, which contains the gene encoding the HLA complex class II HLA-DQ alpha chain 1 (*HLA-DQA1*). These findings expand the role of the HLA system to FSGS suggesting a common pathway for the development of proteinuria across multiple glomerular diseases, including childhood-onset steroid sensitive nephrotic syndrome, FSGS, and membranous nephropathy, as was previously reported.

   We are currently expanding our investigation to a larger cohort that encompasses greater race/ethnicity diversity in order to increase the statistical power to detect significant associations.

Phenotypes	N (%)
Focal Segmenal Glomerulosclerosis (FSGS)	**1545** (78.9)
Minimal Change Disease (MCD)	**149** (7.6)
Nephrotic Syndrome Not Otherwise Specificied (NS-NOS)	**52** (2.7)
IgM Nephropathy (IgMN)	**15** (0.8)
Proliferative Mesangial Glomerulonephritis (PMGN), IgMN	**81** (4.1)
Proliferative Mesangial Glomerulonephritis (PMGN), IgA excluded	**111** (5.7)
C1q Nephropathy (C1qN)	**4** (0.20)
Gender	**N (%)**
Male	**1154** (58.9)
Female	**803** (41.1)
Total	**1,957**

Table 1. Summary of the clinical phenotypes and gender proportion of patients initially selected for GWAS (N=1,957).

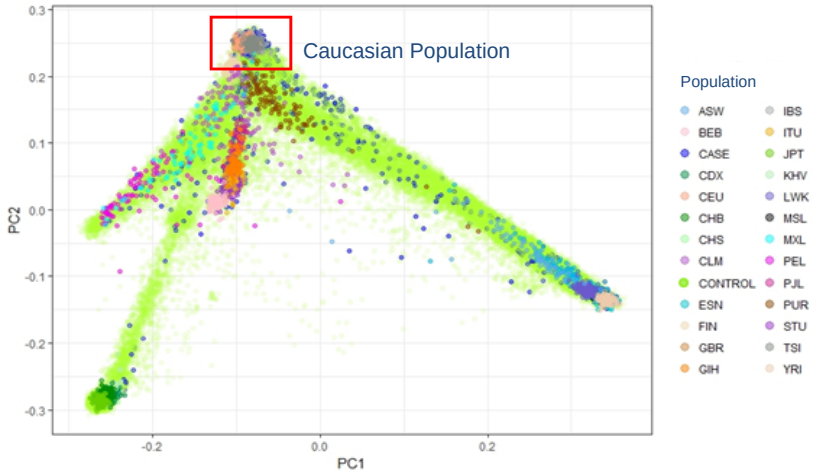

Figure 1. PCA of (patient =1,957) and (control=57,327) cohorts revealing ancestral origins based on 1000 genome project samples.

Phenotypes	N (%)
Focal Segmenal Glomerulosclerosis (FSGS)	**874** (75.8)
Minimal Change Disease (MCD)	**103** (8.9)
Nephrotic Syndrome Not Otherwise Specificied (NS-NOS)	**2** (0.17)
IgM Nephropathy (IgMN)	**6** (0.52)
Proliferative Mesangial Glomerulonephritis (PMGN), IgMN	**67** (5.8)
Proliferative Mesangial Glomerulonephritis (PMGN), IgA excluded	**101** (8.8)
Gender	**N (%)**
Males	**655** (56.8)
Females	**498** (43.2)
Total	**1,153**

Table 2. Summary of the clinical phenotypes and gender proportion of Caucasian patients comprising the three cohorts selected for GWAS (N=1,153).

	Allele Count (Frequency %)		Genotype Count (Frequency %)				Additive Association Test Results			
	G	A	GG	AG	AA	O(2pq Het Freq) %	Odds Ratio	Standard Error	CI% (L95-U95)	*P*- value
Cases (1153)	472 (20.5)	1834 (79.5)	43 (3.7)	386 (33.5)	724 (62.8)	32.6	1.57	0.065	(1.29-1.67)	1.50E-08
Controls (2392)	715 (14.9)	4069 (85.1)	66 (2.8)	583 (24.4)	1743 (72.8)	25.4				

Table 3. Association of alleles and genotypes of rs28383303 on chr.6 following a meta-analyzed regression association test.

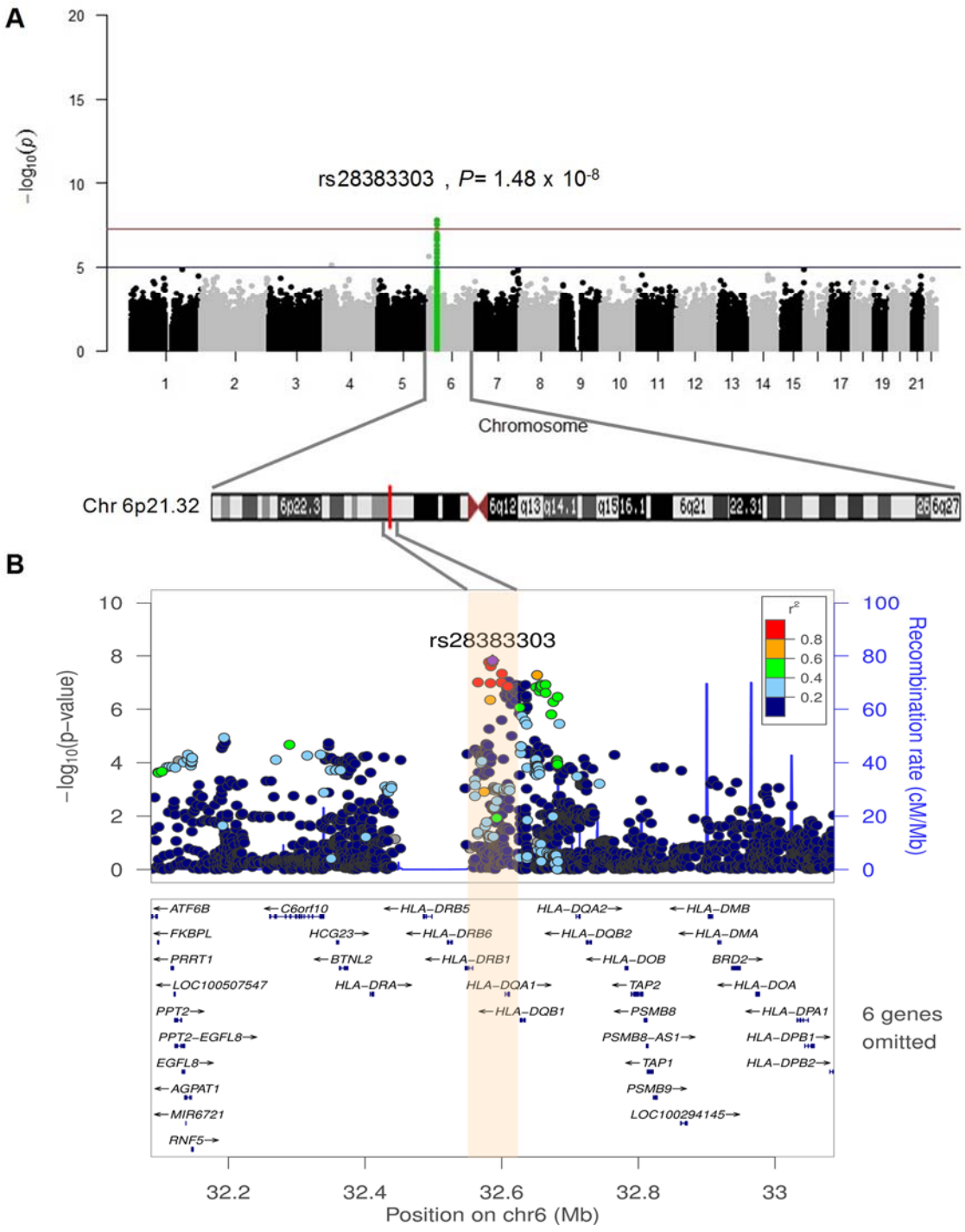

Figure 2: Results of GWAS meta-analysis on 1,153 cases and 2,392 controls. (**A**) Manhattan plot of the genome-wide *P* values for each SNP. rs28383303 surpassed the genome-wide significance threshold ($P= 1.48 \times 10^{-8}$). (**B**) Regional plot of the 6p21.32 for the combined genome-wide association results. The blue lines represent recombination rates. rs28383303 is designated by a diamond and all other SNPs by a circle. The 50kb haploblock contains the genes *HLA-DQA1 and HLA-DRB1.*

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

- This award currently provides full support for a postdoctoral scientist in my lab with the opportunity to identify and promote her skill set as part of her training experience. As part of her training she has one-on-one meetings with me on a weekly base for both scientific and career development; she has partnership and additional mentorship with bioinformaticians and statistical geneticists (Dr Ionita-Laza) to foster her training in programming and statistics; she is engaged in presenting her results to national and international meeting as well as manuscript preparation.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The preliminary interim GWAS analysis has been submitted for presentation to the following meetings: Human Genetics in New York (Sept 12 2017); Italian Society of Nephtology (Oct 3-7 2017); American Society of Nephrology (Nov 1-5 2017).

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

1. Complete sample collection and microarray SNP genotyping for all newly employed samples.
2. Conduct a GWAS to identify common, risk-conferring variants using the entire patient cohort genotyped thus far. This will entail genotype imputation and data harmonization across populations and platforms.
3. Perform a CNV burden analysis and annotation for large, rare CNVs that underlie nephrotic syndrome variants in our current patient cohort.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Year 1 of funding has mostly dedicated to data collection and generation, but the interim analysis on a small fraction of the propose dataset strongly indicate that there are common variants with relatively large effect size for NS implicating that this study will have high impact on understating the genetic predisposition and biology of kidney disease and FSGS in specific.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*

- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

1. Ahram DF., [et al, including Gharavi AG, Kiryluk K, Ghiggeri GM, Hildebrandt F, Sampson MG, Sanna-Cherchi S]. HLA Alleles Confer Risk to Primary Idiopathic Nephrotic Syndrome in Individuals of Caucasian Ancestry. American Society of Nephrology (ASN). (2017) (Poster).

2. Ahram DF., [et al, including Gharavi AG, Kiryluk K, Ghiggeri GM, Hildebrandt F, Sampson MG, Sanna-Cherchi S]. HLA Alleles Confer Risk to Primary Idiopathic Nephrotic Syndrome in Individuals of Caucasian Ancestry. Italian Society of Nephrology (ISN). (2017) (Poster).

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: *Simone Sanna-Cherchi*
Project Role: *Principal Investigator*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *2.4*

Contribution to Project: *Study design and conceptualization, mentoring of postdoc and technician, data dissemination.*
Funding Support: Dr. Sanna-Cherchi' s funding portfolio currently includes NIH 1R01DK103184-01 and Mentor role for ASN Foundation for Kidney Research Ben J.Lipps Research Fellowship ASN CU16-1275

Name: *Dina Ahram*
Project Role: *Post Doc*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *12.0*

Contribution to Project: *Study design and data analysis, supervision of wetlab experiments, statistical analyses, preparation of results for presentations*
Funding Support: *N/A*

Name: *Qingxue Liu*
Project Role: *Technician*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *4.8*

Contribution to Project: *Wet lab experiments, DNA preparation and plating*
Funding Support: *N/A*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

At the time of the grant submission David A. Fasel was the main analyst for this project (100% effort) but Dr. Dina Ahram substituted David Fasel from the beginning of the funding period with the same 100% effort.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.